REMARKS

Claims 78-92 are pending. Claim 83, 85 and 86 have been amended to change the dependency. Claim 93 has been added to recite antibodies, as opposed to antibody or antibody fragment. Claims 78-93 remain in the case.

Applicant respectfully requests that the foregoing amendments be made prior to examination of the present application, and respectfully requests reconsideration of the present application in view of the foregoing amendments and the reasons that follow. This amendment adds, changes and/or deletes claims in this application. A detailed listing of all claims that are, or were, in the application, irrespective of whether the claim(s) remain under examination in the application, is presented, along with appropriate defined status identifiers.

The Examiner has acknowledged applicant's cancellation of priority claims to USSN 07/167,077 and 06/751,877. The examiner requests clarification of the present application to EP 03 07 6875 and EP 03 07 6876. These two European applications are counterparts of the present application, but claim other aspects of the invention disclosed in the present application. The former relates to a method and kit for imaging organs and tissues using labelled antibodies, whereas the latter relates to a method for affecting cellular function using antibodies. The IDS submitted February 9, 2004, included documents that were cited in the European search reports for these two applications. Applicants are now providing copies of the cited foreign patent documents on the PTO/SB/08 filed February 9, 2004, with the exception of EP 0 330 201, which was submitted and considered on Form PTO-1449 filed December 5, 2001. The articles listed on the IDS all were cited in the European Search Report for EP 03 07 6875, which relates to imaging. Four of these articles were made of record in the IDS submitted on December 5, 2001, including the two documents that relate to imaging of bone marrow (Duncker and Reske). The other cited documents are less relevant than these. Applicants request an initialed copy of Form SB/08 indicating the Examiner's consideration of the cited foreign patent documents.

The Examiner questions applicant's claims for domestic priority based on USSN 09/110,181 and USSN 07/866,789. Specifically, the Examiner alleges that there is insufficient support for the term "marker associated with a B cell."

The specification describes that a "marker" is an entity to which an antibody or antibody fragment binds. Thus, page 15, lines 8-9 references that "the antibody is an antibody or

antibody fragment which specifically binds a ***marker*** produced by or associated with said cell or tissue." Page 5, lines 19 and 20 describe "an antibody or antibody fragment specific to a marker associated with or produced by bone marrow cells," and page 12, lines 12-16 describes "antibodies and fragments against bone marrow cells, particularly hematopoietic progenitor cells, pancreatic islet cells, spleen cells, parathyroid cells, uterine endometrium, ovary cells, testicular cells, thymus cells, B-cells..." Page 12, lines 30-33 discloses that "Antibodies that target the spleen well include the LL2 (also known as EPB-2) monoclonal antibody, disclosed in Pawlak-Byczkowska, *Cancer Research*, 49:4568-4577 (1989), which is directed against normal and malignant B-cells." Therefore, the parent applications USSN 09/110,181 and USSN 07/866,789 clearly support the term "marker associated with a B cell." Therefore, the present application properly claims priority to USSN 09/110,181 and 07/866,789, the latter of which was filed April 7, 1992.

The examiner has objected to the title as not being descriptive of the invention being claimed. A new title has been provided on page 1 of the application. As requested, the specification also has been amended to indicate trademarks.

Claims 83-92 are rejected under the second paragraph of Section 112 as being indefinite. The dependencies of claims 83, 85 and 86 have been corrected to obviate this rejection.

Claims 87-92 are rejected under the first and second paragraphs of Section 112 based on their recitation of "LL2 antibody." The Examiner urges that LL2 antibody is "merely a laboratory designation which does not clearly define the claimed product." LL2 is an anti-CD22 antibody. It is available to the public and its scope is clearly understood by the knowledgeable reader. Indeed, it has been used in the claims of several issued US patents without any reference to SEQ IDs or Deposit Accession Number, including US 6,395,276, US 6,653,104, US 6,306,393, US 6,183,744, and US 6,846,476, demonstrating both its availability and its well-understood scope. Reconsideration and withdrawal of this ground of rejection is respectfully requested.

In paragraph 10 of the Action, claims 78-92 are rejected under the first paragraph of Section 112, as failing to comply with the written description requirement. The Examiner urges that the term "a marker associated with a B cell" is not supported by the disclosure as filed, stating that "the specification only discloses LL2 monoclonal antibody that target the spleen cell;

the instant claims now recite any antibody specific to a marker associated with a B cell, which were not clearly disclosed in the specification." She goes on to state that applicant relies upon a "generic disclosure of antibodies and possibly a single or limited species." However, the specification includes more than a disclosure of "antibodies" and "LL2 antibody," which is a species of B-cell targeting antibody. It more broadly discloses "an antibody or antibody fragment specific to a marker associated with or produced by bone marrow cells," (page 5, lines 19 and 20) and "***antibodies and fragments against*** bone marrow cells, particularly hematopoietic progenitor cells, pancreatic islet cells, spleen cells, parathyroid cells, uterine endometrium, ovary cells, testicular cells, thymus cells, ***B-cells***..." (page 12, lines 12-16). Accordingly, "an antibody or antibody fragment specific to a marker associated with a B cell" were clearly disclosed in the parent specification and the present claims are not a "departure" from the specification and claims as originally filed. Reconsideration and withdrawal of the rejection for lack of written description/new matter is respectfully requested.

In paragraph 11 of the Action, claims 78-92 are again rejected under the first paragraph of Section 112, this time as failing to comply with the written description requirement. The Examiner urges that "the claims recite a genus 'an antibody or antibody fragment specific for a marker associated with a B cell' as part of the invention without providing a physical structure or testable functional activity for the 'a marker associated with a B cell'" (emphasis in original). However, a skilled artisan can readily determine whether an antibody or antibody fragment is specific for a marker associated with a B cell. There exist cultured cell lines that express various B cell antigens. A skilled artisan readily can assess whether an antibody binds to the B cell antigen on these cell lines, and therefore there is a testable functional activity associated with the term "specific for a marker associated with a B cell." For example, Stein *et al.*, *Cancer Immunol Immunother*, 1993, 37(5):293-8 (abstract appended), describes studies in which the specificity of LL2 was determined by binding studies with cultured cell lines, Nalm-6 and Molt-4. The binding profile of LL2 on these cell lines was consistent with anti-CD22, but not anti-CD21. Sequential immunoprecipitation and cross-blocking studies with anti-CD22 monoclonal antibodies recognizing established CD22 epitopes were performed to confirm that LL2 reacts with CD22 and to determine which epitope LL2 recognizes. Antibodies to other B cell markers can similarly be "tested" with cultured cell lines that express other of the B cell markers, such as CD19 and CD20, and thus antibodies to any of the B cell markers have a testable functional activity and hence are fully described.

The Examiner goes on to state that "applicant has disclosed only a monoclonal LL2 antibody that targets the spleen cell." However, the LL2 antibody is a B cell antibody and shares the characteristic of all "B cell antibodies" which is the ability to bind specifically to B cells. The LL2 antibody does also bind to normal spleen tissue, as explained in the following excerpt from Coleman, *et al.* (*Clinical Cancer Research*, Vol. 9, 3991S-3994S, September 1, 2003, a copy of which is appended):

> Epratuzumab is a humanized IgG1 monoclonal antibody directed against the CD22 antigen. Its parent murine antibody (LL2) has a broad range of reactivity against various B-cell lymphoma subtypes as demonstrated by immunohistochemistry (48) , with little binding to normal tissue except for spleen. The antibody is rapidly internalized after attachment to CD22 (34) . The murine LL2 antibody was subsequently re-engineered into the humanized (hLL2) epratuzumab (16).

Accordingly, the statement in the specification that LL2 antibody targets the spleen cell is a known fact and in no way detracts from its status as a characteristic B cell antibody.

The Examiner further urges that "an antibody fragment can be any one of the constant regions (CH1-3) and also may be the hinge region. However, the language also reads on small amino acid sequences which are incomplete regions of the constant region of the antibody." In reply, applicant notes that the claims recite an antibody or antibody fragment that is "specific" to a marker associated with a B cell. The constant regions, antibody fragments that are the hinge region, and small amino acid sequences where are incomplete regions of the constant region of the antibody will not be "specific" to a B cell marker. Accordingly, the non-specific entities identified by the Examiner would not be encompassed within the scope an antibody or antibody fragment "specific" to a marker associated with a B cell.

A skilled artisan knows of many B cell antigens (or markers), and could make antibodies to any of these antigens. There is no need for the application to go into detail describing how to make antibodies to various B cell antigens, this is within the level of skill in this art. Nor is there any need for the skilled artisan to know the exact structure of the antibodies so-produced. Applicant's contribution is the teaching that antibodies to B cell markers are useful in the treatment of immune diseases. Armed with this teaching, the skilled artisan could make antibodies to any of the well-described B cell antigens and use these to treat immune diseases without understanding any more about their structure. Isolation and characterization are ***not***

required, as alleged by the Examiner at the top of page 11. The feature that such antibodies possess in common is the ability to bind specifically with a marker associated with a B cell. This feature allows one skilled in the art "to visualize or recognize the identity of the subject matter of the claim."

Claims 78-92 are rejected under Section 102(b) based on Goldenberg (WO93/19668) as evidenced by Goldenberg (US Patent 6,183,744). WO93/19668 is the parent of the present application, and as demonstrated above, the presently claimed method of method of treating an immune disease using antibody or antibody fragment specific to a marker associated with a B cell is fully supported in this parent. Indeed, it is not understood how the Examiner can, on the one hand, urge that this parent does not support the teaching of a method of treating immune disease using a B cell antibody and then cite it under Section 102 as defeating novelty of the present claims. Perhaps it is because the Examiner is alleging that it was not known that LL2 antibody is specific for B cells until later, the examiner citing applicant's later-filed application that issued as US 6,183,744. However, the fact that LL2 was specific for a B cell marker was known before the 1992 filing date of the present application, as evidenced by Goldenberg *et al.* (*J Clin Oncol*, 1991 Apr, 9(4):548-64). The abstract of this document is appended and states that "LL2 is a murine IgG2a monoclonal antibody (MAb) reactive with B cells and non-Hodgkin's B-cell lymphoma."

Furthermore, as noted above, applicant is not relying merely on the disclosure of LL2 as supporting the recitation of antibodies specific to a marker associated with a B cell, since the present disclosure clearly stated that the present invention included "antibodies and fragments against bone marrow cells, particularly hematopoietic progenitor cells, pancreatic islet cells, spleen cells, parathyroid cells, uterine endometrium, ovary cells, testicular cells, thymus cells, B-cells..." Based on the foregoing, reconsideration and withdrawal of the rejection based on Goldenberg (WO93/19668) as evidenced by Goldenberg (US Patent 6,183,744) is respectfully requested.

Claims 78-92 are rejected under Section 102(b) based on Hansen *et al.* (US Patent 5,443,953). Hansen *et al.* was filed on December 8, 1993, after the April 7, 1992 filing date to which the present application claims domestic priority. Reconsideration and withdrawal of the rejection based on Hansen *et al.* is respectfully requested.

Claims 78-92 are rejected under Section 102(e) based on Goldenberg *et al.* (US Patent 7,074,403). The provisional application on which US 7,074,403 is based was filed on June 9, 1999, after the April 7, 1992 filing date to which the present application claims domestic priority. Reconsideration and withdrawal of the rejection based on US 7,074,403 is respectfully requested.

If there are any problems with this response, or if the examiner believes that a telephone interview would advance the prosecution of the present application, Applicant's attorney would appreciate a telephone call. In view of the foregoing, it is believed none of the references, taken singly or in combination, disclose the claimed invention. Accordingly, this application is believed to be in condition for allowance, the notice of which is respectfully requested.

Respectfully submitted,

ROSSI, KIMMS & McDOWELL LLP

DECEMBER 18, 2006
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Goldenberg, et al., "Targeting, dosimetry, and radioimmunotherapy of B-cell lymphomas with iodine-131-labeled LL2 monoclonal antibody" *J Clin Oncol.* 1991 Apr;9(4):548-64.

Sixteen patients with non-Hodgkin's lymphoma were infused with 6.2 to 58.2 mCi (0.2 to 3.9 mg) doses of radioactive iodine (131I)-labeled LL2 immunoglobulin G (IgG) or F(ab')2, in order to study antibody distribution, pharmacokinetics, dosimetry, toxicity, tumor targeting, and therapy. LL2 is a murine IgG2a monoclonal antibody (MAb) reactive with B cells and non-Hodgkin's B-cell lymphoma. In a series of five assessable therapy patients, doses as small as 30 mCi 131I-LL2 IgG or F(ab')2 resulted in tumor responses (two partial remissions, two mixed and minor responses, and one no response), while one patient receiving diagnostic doses as low as 6.2 mCi showed a partial remission for 1 year and a complete remission after a second low radiation dose. No acute toxicities were noted, and only myelotoxicity accompanied therapeutic doses, with grade IV marrow toxicity seen in three of seven patients receiving total doses of about 50 mCi. Dosimetry calculations showed spleen and tumor dose rules of about 4.6 cGy/mCi, which was three to four times the dose to other organs. Despite the administration of relatively low doses of LL2 (0.2 to 3.9 mg), 82% of 60 known extrasplenic lymphoma sites were imaged. Serum clearance showed an average distribution half-life (T1/2) of 2.1 hours and an elimination T1/2 of 32.0 hours. The average total-body clearance T1/2 was 43 to 45 hours. LL2's antigenic target does not appear to be shed in high amounts into the circulation. Three of eight patients having at least two injections showed a human antimouse antibody response. These patients may have been presensitized to animal protein. An interesting observation in this study was the marked drop in circulating B lymphocytes after the administration of radioiodinated LL2 or anticarcinoembryonic antigen MAbs, suggesting that this is a nonspecific radiation effect and not necessarily related to the binding of MAb to normal B cells.

Stein *et al.*, "Epitope specificity of the anti-(B cell lymphoma) monoclonal antibody, LL2," *Cancer Immunol Immunother.* 1993 Oct;37(5):293-8

LL2 is a murine monoclonal antibody IgG2a reactive with B cells and non-Hodgkin's B-cell lymphoma, which, in a radioiodinated form, induces responses in lymphoma patients [Goldenberg et al. (1991) *J Clin Oncol* 9:548-564]. In this report we identify LL2 as a member of the CD22 cluster. The molecular size of the antigen, its expression profile, and competitive blocking studies were used to establish this identification. By Western blot analysis and immunoprecipitation studies using the Raji Burkitt's lymphoma cell line metabolically labelled with [3H]leucine, the LL2 antigen was determined to correspond to a molecular mass of 140 kDa. The molecular mass of the LL2 antigen, and the B-cell-restricted reactivity of the LL2 antibody, were consistent with both the CD21 and CD22 clusters. To assess additional similarities and differences between LL2 and anti-CD22 and anti-CD21, the binding of these mAb to cultured cell lines, Nalm-6 and Molt-4, was compared by flow cytometry. The binding profile of LL2 on these cell lines was consistent with anti-CD22, but not anti-CD21. Sequential immunoprecipitation and cross-blocking studies with anti-CD22 monoclonal antibodies recognizing established CD22 epitopes were performed to confirm that LL2 reacts with CD22 and to determine which epitope LL2 recognizes. Binding of 131I-LL2 to Raji cells is inhibited over 90% by prior incubation of the target cells with unlabelled RFB4, indicating that LL2 belongs to the same epitope group as RFB4, *i.e.*, epitope B.

Epratuzumab: Targeting B-Cell Malignancies through CD22[1]

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Abstract

The development of effective B cell-directed monoclonal antibody therapies has dramatically altered the management of patients with B-cell non-Hodgkin's lymphoma. Anti-CD20 murine and chimeric antibodies have been characterized by manageable toxicity profiles and appear to have mechanisms which may be distinct from and complementary to those of chemotherapy. There is considerable rationale for treatment strategies which target other B-cell antigens, including CD22. This molecule is commonly expressed in non-Hodgkin's lymphoma and may mediate important functions in B-cell biology. Laboratory and initial clinical studies suggest that epratuzumab, a humanized anti-CD22 monoclonal antibody, may have antilymphoma activity in both unlabeled and radiolabeled forms. Efforts are underway to establish the utility of epratuzumab as a treatment for B-cell malignancies, through single agent and combination regimens, to define the optimal settings for its clinical application.

Introduction

NHL[3] is comprised of a heterogeneous group of malignancies which all represent neoplasms of lymphocytes. Although numerous NHL subtypes have been recognized, the most commonly observed entities are FL and LCL.

Chemotherapy, along with radiotherapy, has served as the mainstay of treatment for decades. The first significant departure from exclusive reliance on these therapeutics came from the FDA approval and introduction of rituximab, an unlabeled ("naked" or "cold") chimeric monoclonal antibody directed toward the CD20 antigen, which is anchored to the surface of most B-cell lymphoma cells. The demonstration that rituximab could produce ≥60% objective responses in low-grade NHL patients (depending on histology and previous therapy) with minimal toxicity has significantly impacted clinical practice and sparked enormous enthusiasm for this new modality of therapy (1–7). Subsequent studies have suggested that rituximab may be easily combined either sequentially or concurrently with chemotherapy, with acceptable side effects and potentially improved efficacy (8–11).

Although rituximab has proven especially beneficial to FL patients, a substantial proportion of patients do not respond, and almost every responding patient ultimately relapses, usually at a median of 9–12 months. Nevertheless, rituximab, representing proof of principle for the clinical application of antibodies in NHL, has prompted the search for new antibodies directed to other B cell lineage-specific antigen targets which may exhibit different patterns of tumor expression or may be associated with biological properties distinct from those of CD20.

CD22 as a Target for Immunotherapy

A variety of lymphocyte antigens is currently under evaluation as targets for immunotherapy. These include CD22, CD52, HLA-DR, CD80, and CD30. All have preliminarily exhibited encouraging results in preclinical or clinical studies (12–28). It has been hypothesized that antibodies against other lymphoma antigens may have antilymphoma effects that could overcome rituximab resistance or augment the activity of rituximab.

The CD22 antigen is a M_r 135,000 B-lymphocyte-restricted transmembrane sialoglycoprotein of the immunoglobulin superfamily. The predominant CD22 isoform contains seven extracellular domains (29, 30). CD22 is initially present in the cytoplasm of developing B cells but is later expressed on the surface during B-cell maturation once IgD expression occurs (31). Most circulating IgM+IgD+ cells express CD22. CD22 is strongly expressed in follicular (primary and secondary, B-cell zones), mantle, and marginal zone B cells but is weakly present in germinal (activated or differentiating) B cells. In B-cell malignancies, CD22 has been observed in ≥60–80% of samples evaluated (32). However, limited data are available with regard to expression of different CD22 isoforms in various NHL subtypes. When bound by ligand or antibody, CD22 is rapidly internalized within hours; internalization is "terminal," and re-expression is slow (days) (33, 34). The function of CD22 has not been entirely clarified, but reports have implicated a number of biological activities, including cellular adhesion and homing, as well as regulation of the B-cell activation (31, 35–38). Notably, CD22-deficient mice have mature B cells which may be more susceptible to apoptotic signals, have a shorter cellular life span, and are reduced in number in the bone marrow (39).

Potential Advantages of a Humanized Antibody Structure

Most monoclonal antibodies had been initially generated in a murine form and sometime later were modified to either chimeric (part murine and part human) or humanized forms.

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[1] Presented at the "Ninth Conference on Cancer Therapy with Antibodies and Immunoconjugates," October 24–26, 2002, Princeton, NJ. Supported in part by a K23 award (to J. P. L) from the NIH (RR16814-02) and a pilot grant from the Cornell Center for Aging Research and Clinical Care (to J. P. L.).

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[3] The abbreviations used are: NHL, non-Hodgkin's lymphoma; FL, follicular lymphoma; LCL, large B-cell lymphoma.

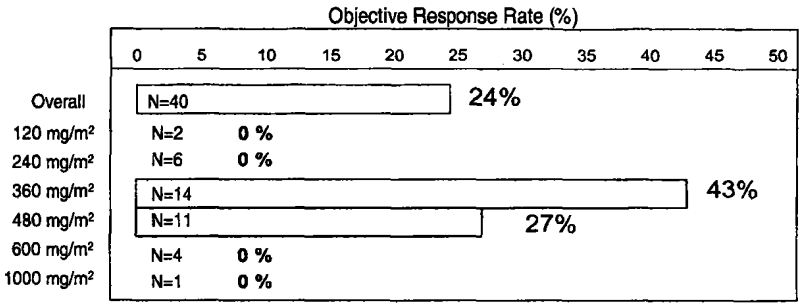

Fig. 1 Objective response rates by dose for evaluable patients with follicular NHL in Phase I/II study of epratuzumab in indolent NHL. The 95% confidence intervals were 11–40%, 18–71%, and 6–61% for the rates for overall, the 360 mg/m^2, and the 480 mg/m^2 groups, respectively. Reproduced from Leonard *et al.* (54) with permission from the authors and publisher.

Favorable characteristics of humanized antibodies may include a more extended half-life, which potentially allows for extended dosing intervals, and reduced immunogenicity, which confers value for multiple dose strategies. These qualities have potentially important relevance to dosing regimens and pharmacokinetics that may affect the therapeutic response and toxicity. Antibody structure may also impact the capability of an individual antibody to mediate effector cell killing through binding to Fc receptors (40). Various investigators are actively developing approaches which optimize antibody structure to improve engagement of the immune system to potentially improve antitumor effects. Other approaches include the addition of radioisotopes or toxins to antibodies to deliver additional modalities, which may also induce cell death (17, 19, 41–47).

Epratuzumab (Humanized LL2)

Epratuzumab is a humanized IgG1 monoclonal antibody directed against the CD22 antigen. Its parent murine antibody (LL2) has a broad range of reactivity against various B-cell lymphoma subtypes as demonstrated by immunohistochemistry (48), with little binding to normal tissue except for spleen. The antibody is rapidly internalized after attachment to CD22 (34). The murine LL2 antibody was subsequently re-engineered into the humanized (hLL2) epratuzumab (16). A number of different studies has been or are being conducted with both murine and humanized radiolabeled (Iodine-131 and Yttrium-90) forms of LL2, as well as with the unlabeled humanized form (epratuzumab; Immunomedics, Morris Plains, NJ, and Amgen, Thousand Oaks, CA). Laboratory investigations of putative mechanisms of action of epratuzumab as antilymphoma therapy are also underway. Antibody-dependent cellular cytotoxicity, as well as other pathways, may potentially be involved, similarly to those implicated in the activity of other therapeutic antibodies (49–52).

Clinical Studies of Unlabeled Epratuzumab (hLL2)

Although initial studies of epratuzumab focused on evaluation of radiolabeled constructs, several characteristics of unlabeled antibodies justify a parallel development strategy. Unlike radiolabeled agents, anti-B cell unlabeled antibodies are generally not associated with myelosuppression, facilitating treatment of patients with pre-existing cytopenias and extensive bone marrow involvement with tumor. Additionally, unlabeled antibodies are easier to combine with chemotherapy and other agents because of nonoverlapping toxicities. These attributes, in addition to the unique properties of the target CD22 antigen, all provided an impetus for the study of unlabeled epratuzumab in B-cell malignancies.

At the Center for Lymphoma and Myeloma at the Weill Medical College of Cornell University and the New York Presbyterian Hospital, we have explored the use of epratuzumab in patients with a variety of relapsed and refractory B-cell malignancies (53). An initial dose escalation trial used epratuzumab at i.v. doses from 120 to $\leq$1000 mg/m^2/week for four treatments, given generally over 30–60 min. Premedication with acetaminophen and diphenhydramine was provided to minimize the potential for infusion reactions. Treated subjects were heavily pretreated, with half having received four or more previous treatment regimens. Other adverse prognostic features were commonly present, including increased lactate dehydrogenase and tumor masses of $\geq$5 cm. Epratuzumab was very well tolerated across all dose levels examined, even when infused over $\leq$1 h. Infusion toxicities have been primarily grade 1 and manageable with usual supportive measures. Some patients exhibited transient B-cell depletion, but no other consistent laboratory abnormalities have been observed. No dose-limiting toxicity was observed, although for logistical reasons, escalation beyond 1000 mg/m^2/week was not performed. Among initial groups of FL, patients treated across all dose levels (n = 40), three complete and six partial responses, were preliminarily observed. Among the first group of LCL patients receiving epratuzumab, five objective responses were noted, including three complete responses. Overall responses appear to be more frequent around the 360 and 480 mg/m^2 dose levels (Fig. 1). Some of the responses have extended as long as several years. The tolerability and clinical activity in FL and LCL have suggested that further evaluation of this agent in NHL is warranted.

This preliminary evidence of antilymphoma activity led our group to study the concomitant use of epratuzumab and rituximab, to our knowledge the first study of combination antibody therapy in lymphoma. Because the mechanisms of action of the two agents may be different, and the targets are distinct, it is possible that the addition of epratuzumab may augment the activity of an anti-CD20-based regimen. Of course, one could theoretically postulate reduced efficacy of a combination, through a deleterious effect, although with disparate targets, a competition effect would be less likely. Additionally, the toxicity profile of a combination immunotherapy strategy

may be potentially more favorable relative than that of chemotherapy or radioimmunotherapy. Preliminary evaluation of the combination of epratuzumab and rituximab in B-cell NHL has yielded encouraging findings (54). Patients have received 360 mg/m^2 epratuzumab followed by 375 mg/m^2 rituximab weekly for four doses. Subjects have predominantly fallen into the FL and LCL categories, and early enrolling patients were rituximab naïve. Combination therapy was well tolerated with infusion-related toxicities National Cancer Institute grade I or II and comparable with those seen with antibody monotherapy. Objective responses have been demonstrated in the majority of patients (preliminarily 66% of follicular patients), and the quality of responses as reflected by complete responses and complete responses unconfirmed (preliminarily 60% in follicular patients) appear to be greater than expected with rituximab alone. Extended follow-up and additional accrual are necessary to validate our initial impressions, but these preliminary results suggest that this combination antibody regimen may be well tolerated and offers encouraging antilymphoma activity.

A number of other clinical trials with epratuzumab are either ongoing or expected, including studies of single agent epratuzumab in various B-cell lymphomas, multicenter evaluation of the epratuzumab and rituximab combination in FL and LCL, and studies of epratuzumab with chemotherapy, such as cyclophosphamide, doxorubicin, vincristine, and prednisone, with rituximab as primary therapy for LCL.

Conclusion and Future Directions

Considerable challenges still remain to elucidate the biology and function of epratuzumab and in the determination of the optimal setting for its use in B-cell malignancies. The evolving clinical data suggest that, ultimately, epratuzumab may have a significant role among the array of therapies available for lymphoma patients and will hopefully contribute to an improved therapeutic outlook for them.

Initial findings appear to indicate that epratuzumab has clinical activity as well as acceptable toxicity in FL and LCL. The unique characteristics of the CD22 target, and the properties of the humanized antibody structure, may contribute to these encouraging effects. Further evaluation of the *in vitro* and clinical characteristics of this treatment approach are under way, including more extended follow-up and additional patients among a variety of clinical studies. Although single agent activity of epratuzumab may be useful, a combination antibody therapy approach is particularly appealing, especially if toxicity is no greater, if the number and/or quality of responses ultimately prove to be better. While over the last several decades investigators have focused on combination chemotherapy strategies for lymphoma, we now have the option to potentially avoid the use of chemotherapy (and its associated toxicities) in certain clinical situations through use of combinations of biological agents, which specifically target malignant cells.

References

1. Maloney, D. G., Liles, T. M., Czerwinski, D. K., *et al.* Phase I clinical trial using escalating single dose infusion of chimeric anti-CD20 monoclonal antibody (IDEC-C2B8) in patients with recurrent B-cell lymphoma. Blood, *84:* 2457–2466, 1994.

2. Maloney, D. G., Grillo-Lopez, A. J., Bodkin, D. J., *et al.* IDEC-C2B8: results of a phase I multiple-dose trial in patients with relapsed non-Hodgkin's lymphoma. J. Clin. Oncol., *15:* 3266–3274, 1997.

3. Maloney, D. G., Grillo-Lopez, A. J., White, C. A., *et al.* IDEC-C2B8: (Rituximab) anti-CD20 monoclonal antibody therapy in patients with relapsed low-grade lymphoma. Blood, *90:* 2188–2195, 1997.

4. McLaughlin, P., Grillo-Lopez, A. J., Link, B. K., *et al.* Rituximab chimeric anti-CD20 monoclonal antibody therapy for relapsed indolent lymphoma: half of patients respond to a four-dose treatment program. J. Clin. Oncol., *16:* 2825–2833, 1998.

5. Piro, L. D., White, C. A., Grillo-Lopez, A. J., *et al.* Extended Rituximab (anti-CD20 monoclonal antibody) therapy for relapsed or refractory low-grade or follicular non-Hodgkin's lymphoma. Ann. Oncol., *10:* 655–661, 1999.

6. Coiffier, B., Haioun, C., Ketterer, N., *et al.* Rituximab (anti-CD20 monoclonal antibody) for the treatment of patients with relapsing or refractory aggressive lymphoma: a multicenter phase II study. Blood, *92:* 1927–1932, 1998.

7. Foran, J. F., Rohatiner, A. Z. S., Cunningham, D., *et al.* European phase II study of rituximab (chimeric anti-CD20 monoclonal antibody) for patients with newly-diagnosed mantle-cell lymphoma, immunocytoma, and small B-cell lymphocytic lymphoma. J. Clin. Oncol., *18:* 317–324, 2000.

8. Cruczman, M., Grillo-Lopez, A., White, C. A., *et al.* Progression-free survival (PFS) after six years (median) follow-up of the first clinical trial of rituximab/CHOP chemoimmunotherapy. Blood, *11:* 2518a, 2001.

9. Coiffier, B., Lepage, E., Briere, J., *et al.* CHOP chemotherapy plus Rituximab compared with CHOP alone in elderly patients with diffuse large B cell lymphoma. N. Engl. J. Med., *346:* 235–242, 2002.

10. Byrd, J. C., Peterson, B. L., Morrison, V. A., *et al.* Randomized phase 2 study of fludarabine with concurrent versus sequential treatment with rituximab in symptomatic, untreated patients with B-cell chronic lymphocytic leukemia: results from Cancer and Leukemia Group B 9712. Blood, *101:* 6–14, 2003.

11. Howard, O. M., Gribben, J., Neuberg, D. S., *et al.* Rituximab and CHOP induction therapy for newly-diagnosed mantle cell lymphoma: molecular complete responses are not predictive of progression-free survival. J. Clin. Oncol., *20:* 1288–1294, 2002.

12. Dyer, M. J. The role of CAMPATH-1H antibodies in the treatment of lymphoid malignancies. Semin. Oncol., *5* (Suppl. 14): 52–57, 1999.

13. Khorana, A., Bunn, P., McLaughlin, P., *et al.* A phase II multicenter study of CAMPATH-1H antibody in previously-treated patients with non bulky non-Hodgkin's lymphoma. Leuk. Lymphoma, *41:* 77–87, 2001.

14. Keating, M. J., Flynn, I., Jain, V., *et al.* Therapeutic role of alemtuzumab (Campath-1H) in patients who have failed fludarabine: results of a large international study. Blood, *99:* 3554–3561, 2002.

15. Rai, K. R., Freter, C. E., Mercier, R. J., *et al.* Alemtuzumab in previously treated chronic lymphocytic leukemia patients who had also received fludarabine. J. Clin. Oncol., *20:* 3891–3897, 2002.

16. Leung, S. O., Goldenberg, D. M., Dion, A. S., *et al.* Construction and characterization of a humanized, internalizing B cell (CD22)-specific, leukemia/lymphoma antibody, LL2. Mol. Immunol., *32:* 1413–1427, 1995.

17. Goldenberg, D. M., Horowitz, J. A., Sharkey, R. M., *et al.* Targeting, dosimetry, and radioimmunotherapy of B-cell lymphoma with iodine-131-labelled LL2 monoclonal antibody. J. Clin. Oncol., *9:* 548–564, 1991.

18. Juweid, M., Sharkey, R. M., Markowitz, A., *et al.* Treatment of non-Hodgkin's lymphoma with radiolabelled murine, chimeric or humanized LL2, an anti-CD-22 monoclonal antibody. Cancer Res., *55:* 5899s–5907s, 1995.

19. Vose, J. M., Colcher, D., Gobar, L., *et al.* Phase I/II trial of multiple dose 131 iodine-Mab LL2 (CD22) in patients with recurrent non-Hodgkin's lymphoma. Leuk. Lymphoma, *38:* 91–101, 2000.

20. Lin, T. S., Stock, W., Lucas, M. S., *et al.* A Phase I dose-escalation study of apolizumab (Hu1D10) using a stepped-up dosing schedule in

patients with Chronic Lymphocytic Leukemia (CLL) and Acute Lymphocytic Leukemia (ALL). Blood, 802a, 2002.

21. Link, B. K., Wang, H., Byrd, J.C., *et al.* Phase I study of Hu1D10 monoclonal antibody (Remitogen ™) in patients with B-cell lymphoma. Proc. Am. Soc. Clin. Oncol., 284a, 2001.

22. Brown, K. S., Levitt, D. J., Shannon, M., *et al.* Phase II trial of Remitogen ™(Humanized 1D10) Monoclonal antibody targeting class II in patients with relapsed low-grade or follicular lymphoma. Clinical Lymphoma, *2:* 188–190, 2002.

23. Nagy, Z. A., Hubner, B., Lohning, C., *et al.* Fully human, HLA-DR-specific monoclonal antibodies efficiently induce programmed death of malignant lymphoid cells. Nat. Med., *8:* 801–807, 2002.

24. Bolognesi, A., Polito, L., Tazzari, P. L. *et al.* In vitro anti-tumor activity of anti-CD80 and anti-CD86 immunotoxins containing type I ribosome-inactivating proteins. Br. J. Hematol., *110:* 351–361, 2000.

25. Hariharan, K., Anderson, D., Leigh, B., *et al.* Therapeutic activity of IDEC-114 (Anti CD80) and rituximab (Rituxan®) in B cell lymphoma. Blood, 608a, 2001.

26. Czuczman, M. S., Witzig, T. E., Younes, A., *et al.* IDEC-114, an anti-CD80 monoclonal antibody for relapsed or refractory, follicular NHL: a phase I/II study of safety, efficacy and pharmacokinestics. Blood, 163a, 2002.

27. Bartlett, N., Younes, A., Carabasi, M. A., *et al.* Phase I study of SGN-30, a chimeric monoclonal antibody (mAb), in patients with refractory or recurrent CD30+ hematologic malignancies. Blood, 362a, 2002.

28. Borchmann, P., Schnell, R., Fuss, I., *et al.* Phase I trial of the novel bispecific molecule H22xKi-4 in patients with refractory Hodgkin lymphoma. Blood, *100:* 3101–3107, 2002.

29. Engel, P., Nojima, Y., Rothstein, D., *et al.* The same epitope on CD22 of B lymphocytes mediates the adhesion of erythrocytes, T and B lymphocytes, neutrophils, and monocytes. J. Immunol., *15:* 4719–4732, 1993.

30. Powell, L. D., and Varki, A. I-type lectins. J. Biol. Chem., *270:* 14243–14246, 1995.

31. Tedder, T. F., Tuscano, J., Sato, S., *et al.* CD22, a B lymphocyte-specific adhesion molecule that regulates antigen receptor signaling. Annu. Rev. Immunol., *15:* 481–504, 1997.

32. Cesano, A., Gayko, U., Brannan, C., Kapushoc, H., Fields, S. Z., and Perkins, S. L. Differential expression of CD22 in indolent and aggressive non-Hodgkin's lymphoma (NHL): implications for target immunotherapy. Blood, 350a, 2002.

33. Shan, D., and Press, O. W. Constitutive endocytosis and degradation of CD22 by human B cells. J. Immunol., *154:* 4466–4475, 1995.

34. Shih, L. B., Lu, H. H., Xuan, H., and Goldenberg D. M. Internalization and intracellular processing of an anti-B cell lymphoma monoclonal antibody, LL2. Int. J. Cancer, *56:* 538–545, 1994.

35. Peaker, C. J., and Neuberger, M. S. Association of CD22 with the B cell antigen receptor. Eur. J. Immunol., *23:* 1358–1363, 1993.

36. Nath, D., van Der Merwe, P. A., Kelm, S., *et al.* The amino-terminal immunoglobulin-like domain of sialoadhesin contains the sialic acid binding site. Comparison with CD22. J. Biol. Chem., *270:* 26184–26191, 1995.

37. Sgroi, D., Koretsky, G. A., and Samenkovic, I. Regulation of CD45 engagement by the B cell receptor CD22. Proc. Natl. Acad. Sci. USA, *92:* 4026–4030, 1995.

38. Kelm, S., Pelz, A., Schauer, R., *et al.* Sialoadhesin, myelin-associated glycoprotein and CD22 define a new family of sialic acid-dependent adhesion molecules of the immunoglobulin superfamily. Curr. Biol., *4:* 965–972, 1994.

39. Nitschke, L., Carsetti, R., Ocker, B., *et al.* CD22 is a negative regulator of B-cell receptor signaling. Curr. Biol., *7:* 133–143, 1997.

40. Clynes, R. A., Towers, T. L., Presta, L. G., *et al.* Inhibitory Fc receptors modulate in vivo cytotoxicity against tumor target. Nat. Med., *6:* 443–446, 2000.

41. Juweid, M., Sharkey, R. M., Markowitz, A., *et al.* Treatment of non-Hodgkin's lymphoma with radiolabelled murine, chimeric or humanized LL2, an anti-CD-22 monoclonal antibody. Cancer Res., *55:* 5899s–5907s, 1995.

42. Linden, O., Tennvall, J., Cavallin-Stahl, E., *et al.* Radioimmunotherapy using 131I-labelled anti-CD22 monoclonal antibody (LL2) in patients with previously-treated B-cell lymphomas. Clin. Cancer Res., *5:* 3287s–3291s, 1999.

43. Juweid, M. E., Stadtmauer, E., Haggar, G., *et al.* Pharmacokinetics, dosimetry and initial therapeutic results with (131I) and (111)IN/90Y-labeled humanized LL2 antI-CD22 monoclonal antibody in patients with relapsed, refractory NHL. Clin. Cancer Res., *5:*3292s–3303s, 1999.

44. Hajjar, G., Burton, J., Sharkey, R. M., *et al.* Phase I/II radioimmunotherapy trial with (90Y)-labelled epratuzumab (LymphoCide; anti-CD22 monoclonal antibody) in relapsed/refractory non-Hodgkin's lymphoma (NHL). J. Nucl. Med., *42s:* 156P, 2001.

45. Linden, O., Tennvall, J., Cavallin-Stahl, E., *et al.* Durable response to 90-yttrium-epratuzumab (hLL2) in B-cell lymphoma failing chemotherapy by using dose-fractionation schedule. Blood, 602a, 2001.

46. Behr, T. M., Wormann, B., Gramatzki, M., *et al.* Low-versus high-dose radioimmunotherapy with humanized anti-CD22 or chimeric anti CD20 antibodies in a broad spectrum of B cell-associated malignancies. Clin. Cancer Res., *5:* 3304s–3314s 1999.

47. Postema, E. J., Mandigers, C. M. P. W., Corstens, F. H. M., *et al.* Final results of a phase I immunotherapy trial using ^{186}Re-epratuzumab for the treatment of patients with non-Hodgkin's lymphoma. Cancer Biother. Radiopharm., *17:* 491, 2002.

48. Pawlak-Byczkowska, E. J., Hansen, H. J., Dion, A. S., and Goldenberg, D. M. Two new monoclonal antibodies, EPB-1 and EPB-2, reactive with human lymphoma. Cancer Res., *49:* 4568–4577, 1989.

49. Byrd, J. C., Kitada, S., Flinn, I. W., *et al.* The mechanism of tumor cell clearance by rituximab in vivo in patients with B-cell chronic lymphocytic leukemia: evidence of caspase activation and apoptosis induction. Blood, *99:* 1038–1043, 2002.

50. Golay, J., Zaffaroni, L., Vaccari, T., *et al.* Biologic response of B lymphoma cells to anti-CD20 monoclonal antibody rituxumab in vitro: CD55 and CD59 regulate complement-mediated cell lysis. Blood, *95:* 3900–3908, 2000.

51. Shan, D., Ledbetter, J. A., and Press, O. W. Apoptosis of malignant human B cells by ligation of CD20 with monoclonal antibodies. Blood, *91:* 1644–1652, 1998.

52. Leonard, J. P., Coleman, M., Matthews, J. C., *et al.* Phase I/II trial of Epratuzumab (humanized anti CD-22 Antibody) in non-Hodgkin's lymphoma. Blood, 358a, 2002.

53. Leonard, J. P., Coleman, M., Ketas, J. C., *et al.* Phase I/II trial of Epratuzumab (humanized anti-CD22 antibody) in indolent non-Hodgkin's lymphoma. J. Clin. Oncol., in press, 2003.

54. Leonard, J. P., Coleman, M., Matthews, J. C., *et al.* Epratuzumab (Anti-CD22) and rituximab (anti-CD20) combination immunotherapy for non-Hodgkin's lymphoma: preliminary response data. Proc. Am. Soc. Clin. Oncol., 266a, 2002.

Research article

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Initial clinical trial of epratuzumab (humanized anti-CD22 antibody) for immunotherapy of systemic lupus erythematosus

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Received: 2 Nov 2005 Revisions requested: 4 Jan 2006 Revisions received: 21 Mar 2006 Accepted: 22 Mar 2006 Published: 21 Apr 2006

Arthritis Research & Therapy 2006, **8**:R74 (doi:10.1186/ar1942)
This article is online at: http://arthritis-research.com/content/8/3/R74

Abstract

B cells play an important role in the pathogenesis of systemic lupus erythematosus (SLE), so the safety and activity of anti-B cell immunotherapy with the humanized anti-CD22 antibody epratuzumab was evaluated in SLE patients. An open-label, single-center study of 14 patients with moderately active SLE (total British Isles Lupus Assessment Group (BILAG) score 6 to 12) was conducted. Patients received 360 mg/m^2 epratuzumab intravenously every 2 weeks for 4 doses with analgesic/antihistamine premedication (but no steroids) prior to each dose. Evaluations at 6, 10, 18 and 32 weeks (6 months post-treatment) follow-up included safety, SLE activity (BILAG score), blood levels of epratuzumab, B and T cells, immunoglobulins, and human anti-epratuzumab antibody (HAHA) titers. Total BILAG scores decreased by ≥ 50% in all 14 patients at some point during the study (including 77% with a ≥ 50% decrease at 6 weeks), with 92% having decreases of various amounts continuing to at least 18 weeks (where 38% showed a ≥ 50% decrease). Almost all patients (93%) experienced improvements in at least one BILAG B- or C-level disease activity at 6, 10 and 18 weeks. Additionally, 3 patients with multiple BILAG B involvement at baseline had completely resolved all B-level disease activities by 18 weeks. Epratuzumab was well tolerated, with a median infusion time of 32 minutes. Drug serum levels were measurable for at least 4 weeks post-treatment and detectable in most samples at 18 weeks. B cell levels decreased by an average of 35% at 18 weeks and remained depressed at 6 months post-treatment. Changes in routine safety laboratory tests were infrequent and without any consistent pattern, and there was no evidence of immunogenicity or significant changes in T cells, immunoglobulins, or autoantibody levels. In patients with mild to moderate active lupus, 360 mg/m^2 epratuzumab was well tolerated, with evidence of clinical improvement after the first infusion and durable clinical benefit across most body systems. As such, multicenter controlled studies are being conducted in broader patient populations.

Introduction

Systemic lupus erythematosus (SLE) is a prototypic autoimmune disease that can involve many organ systems [1]. In Europe and the United States, estimates of the number of affected individuals range from 24 to 65 cases per 100,000 people [1,2]. The clinical course of SLE is episodic, with recurring activity flares causing increasing disability and organ damage. Cyclophosphamide, azathoprine, and corticosteroids remain important for long-term management of most patients having active disease, and even those in clinical remission [1]. Despite the important advances made with these drugs, especially cyclophosphamide, in controlling lupus disease activity, they have considerable cytotoxicity and cause, for example, bone marrow depression, ovarian failure, enhanced risk of bladder cancer, as well as the known side effects of long-term systemic corticosteroid therapy. As such, there continues to be a need for the development of targeted and less toxic therapies.

BCR = B cell antigen receptor; BILAG = British Isles Lupus Assessment Group; HACA = human anti-chimeric antibody; HAHA = human anti-human (epratuzumab) antibody; NCI CTC = National Cancer Institute Common Toxicity Criteria; NHL = non-Hodgkin lymphoma; SLE = systemic lupus erythematosus.

Specific autoantibodies against nuclear, cytoplasmic, and membrane antigens remain the serological hallmark of SLE. While lymphopenia is common, there is an increase in the level of activated B cells [3,4] and characteristic alterations of B cell subpopulations [5,6] that may be driven by extrinsic or intrinsic factors. B cells appear to have a key role in the activation of the immune system, in particular through the production of cytokines and by serving as antigen-presenting cells (reviewed recently in [7]). Although B cell activation can occur independently of T cell help in lupus, a substantial fraction of B cells is activated in a T cell dependent manner [8-10], as demonstrated by isotype switching and affinity maturation of B cells [11,12] and enhanced CD154-CD40 interactions [13]. Useful insight into the pathogenesis of lupus has been obtained with animal models. MRL/lpr mice spontaneously develop a lupus-like autoimmune disease in an age-dependent manner, including autoantibody production, arthritis, skin lesions, and severe nephritis, which usually leads to early demise from renal failure [14]. When rendered B cell deficient, they no longer develop nephritis, mononuclear infiltrates are no longer detectable in the kidneys or skin, the number of activated memory T cells are markedly reduced, and infusions of pooled serum from diseased MRL/lpr mice lead to glomerular antibody deposition, but not the development of renal disease [15,16]. However, when reconstituted with B cells not able to secrete circulating antibodies, they develop nephritis and vasculitis [17]. As such, it appears that B cells play a direct role in promoting disease beyond the production of autoantibodies [18].

Depleting B cells with anti-CD20 monoclonal antibodies has emerged as a potentially new therapeutic strategy for certain autoimmune diseases. The chimeric monoclonal antibody rituximab depletes B cells by targeting the pan-B cell surface antigen CD20. Preliminary experience with rituximab in about 100 patients with SLE (recently reviewed in [7]) and other autoimmune diseases has been encouraging [6,19-22].

Due to the central role of B cells in the pathogenesis of certain autoimmune diseases, targeted anti-B cell immunotherapies would be expected to offer therapeutic value in the setting of SLE. In addition to CD20, another unique target is CD22, a 135 kDa glycoprotein that is a B-lymphocyte-restricted member of the immunoglobulin superfamily, and a member of the sialoadhesin family of adhesion molecules that regulate B cell activation and interaction with T cells [23-27]. CD22 has seven extracellular domains and is rapidly internalized when cross-linked with its natural ligand, producing a potent costimulatory signal in primary B cells [25,28-30]. The function of CD22 in cell signaling is suggested by six tyrosine and three inhibitory domain sequences in the intra-cellular cytoplasmic tail. These inhibitory domains are phosphorylated by the nonreceptor kinase Lyn upon B cell antigen receptor (BCR) activation by IgM ligation, leading to the activation and recruitment of SHP-1 phosphatase [31,32]. SHP-1 is a tyrosine phosphatase that negatively regulates several intracellular signaling pathways, including the calcium pathway, through dephosphorylation of signaling intermediates, such as Lyn and Syk. CD22 is first expressed in the cytoplasm of pro-B and pre-B cells, and then on the surface of B cells as they mature, with expression ceasing with B cell differentiation into plasma cells [23]. Studies in CD22-deficient mice and in CD22-negative cell lines have shown an increase in calcium response to BCR ligation [33-36], indicating that CD22 inhibition of BCR signaling is achieved through the mechanism of controlling calcium efflux in B cells. It has been reported that this effect of CD22 is mediated by potentiation of plasma membrane calcium-ATPase and requires SHP-1 [37]. Animal experiments indicate that CD22 plays a key role in B cell development and survival, with CD22-deficient mice having reduced numbers of mature B cells in the bone marrow and circulation, and with the B cells also having a shorter life span and enhanced apoptosis [31].

Therefore, CD22 is an attractive molecular target for therapy because of its restricted expression; it is not exposed on embryonic stem or pre-B cells, nor is it normally shed from the surface of antigen-bearing cells. Initially, a mouse monoclonal antibody (mLL2, formerly EPB-2) was developed and characterized that specifically binds to the third domain of CD22 [38,39]. Immunohistological evaluation revealed that it recognized B cells within the spleen and lymph nodes, but did not react with antigen unrelated to B cells in normal and solid tumor tissue specimens, and flow cytometry showed no reactivity with platelets, red blood cells, monocytes, and granulocytes in normal peripheral blood [38,39]. The complementarity-determining regions of mLL2 were subsequently grafted onto a human IgG_1 genetic backbone [40]. Epratuzumab, the resulting complementarity-determining region-grafted (recombinant) 'humanized' monoclonal antibody (hLL2), is 90% to 95% of human origin, thus greatly reducing the potential for immunogenicity. Epratuzumab has been shown to mediate antibody-dependent cellular cytotoxicity *in vitro*[41] , and may also exhibit biological activity through modulating BCR function (J Carnahan, R Stein, Z Qu, K Hess, A Cesano, HJ Hansen, DM Goldenberg, manuscript submitted).

In clinical trials, over 400 patients with non-Hodgkin lymphoma (NHL) or other B cell malignancies have received epratuzumab administered as 4 consecutive weekly infusions over about 60 minutes. An initial phase I/II study administered doses of up to 1,000 mg/m^2, with patients premedicated each week with oral acetaminophen and diphenhydramine to minimize potential infusion reactions. Epratuzumab toxicity consisted primarily of mild to moderate transient infusion-related events during the first infusion, and only one patient with a prior right lung resection for a fungal abscess had a serious event (bronchospasm during infusion), which was treated with parenteral medications. Based on this safety record, objective evidence of tumor

response, and less severe depression of circulating B cells [42,43], 4 consecutive weekly doses of 360 mg/m^2 epratuzumab was selected as a sufficiently safe and efficacious treatment regimen to warrant further clinical development. A pharmacokinetic analysis of weekly dosing subsequently demonstrated that the post-treatment serum half-life of epratuzumab in NHL patients was 19 to 25 days, consistent with the half-life of a human IgG_1 [44]. As such, a longer interval between doses was indicated, and a biweekly dosing schedule was selected for this initial study in SLE. We report here the first experience of treating an autoimmune disease with a CD22 antibody, epratuzumab.

Materials and methods

This initial, phase II, open-label, non-randomized, single-center study was undertaken to obtain preliminary evidence of therapeutic activity in SLE, to confirm the safety, tolerance and lack of immunogenicity of epratuzumab in this population, and to evaluate pharmacokinetic and pharmacodynamic parameters. The study was approved by the Ethics Committee of Charité University Hospital.

Patient population

Males or non-pregnant, non-lactating females, ≥ 18 years of age, were eligible to participate provided they had a diagnosis of SLE according to the American College of Rheumatology revised criteria (fulfilled ≥ 4 criteria), with SLE for at least 6 months, and at least one elevated autoantibody level (antinuclear antibodies/ANA and/or anti-dsDNA) and moderately active disease (a score of 6 to 12 for total British Isles Lupus Assessment Group (BILAG) disease activity) at study entry. Patients were excluded if they had prior rituximab or other antibody therapy, allergies to murine or human antibodies, experimental therapy within 3 months, active severe CNS (central nervous system) lupus, laboratory abnormalities (hemoglobin < 8.0 g/dl, WBC (white blood cells) < 2,000/mm^3, ANC (absolute neutrophil cells) < 1,500/mm^3, platelets < 50,000/μl, liver transaminases or alkaline phosphatase more than twice upper limit of normal, serum creatinine > 2.5 mg/dl, or proteinuria > 3.5 gm/day), thrombosis, drug or alcohol abuse, infection requiring hospitalization within 3 months, long-term active infectious diseases (tuberculosis, fungal infections) within 2 years, malignancy (except basal cell carcinoma, cervical carcinoma in situ (CIS), history of recurrent abortions (2 or more), or known HIV, hepatitis B or C, or other immunosuppressive states.

Concomitant medications

Pulsed methylprednisolone, other high-dose corticosteroids, cyclophosphamide, and intravenous, joint, or intramuscular corticosteroid injections were not allowed during the study or within four weeks of study entry. Low-dose corticosteroids (prednisone, = 20 mg/day or equivalent) or background therapy with standard antirheumatic immunosuppressives (for example, azathioprine, methotrexate) was permitted provided there were no dosing changes during the study or within four weeks prior to study entry. Antimalarials, non-steroidal anti-inflammatory drugs (NSAIDs), ACE-inhibitors or angiotensin receptor antagonists were also allowed, provided there were no dosing changes during the study or within two weeks of study entry.

Treatment schedule

After satisfying eligibility, signing informed consent, and undergoing baseline evaluations, all patients received 4 doses of 360 mg/m^2 epratuzumab administered every other week with paracetamol (acetaminophen) and an antihistamine (but no steroids) given as premedication prior to each dose.

Study evaluations

The BILAG system was used to categorize the severity level of lupus disease activity in each patient at study entry and at post-treatment evaluations obtained at 6 (24 hours after the last infusion), 10 and 18 weeks and at an additional 32 weeks (6 month post-treatment) follow-up visit. The BILAG system organizes lupus-associated signs and symptoms according to eight body systems: general/constitutional, mucocutaneous, neurological, musculoskeletal, cardiovascular/respiratory, vasculitic, renal, hematological domains [45,46]. At each evaluation, the presence and change of any signs and symptoms were recorded and the level of any disease activity within each body system determined on a treatment-intent basis, according to BILAG rules as: A (severely active disease sufficient to require disease-modifying treatment, for example, > 20 mg/d prednisolone, immunosuppressants/cytoxics); B (moderately active disease requiring only symptomatic therapy, for example < 20 mg/d prednisolone, antimalarials, NSAIDs alone or in combination); or C (stable mild disease with no indication for changes in treatment). To assign an overall disease activity level for each patient, a total BILAG score was determined by adding a numerical severity score (A = 9, B = 3, C = 1, no activity = 0) across the eight body systems. Other evaluations at these times included an SLE panel (autoantibodies, C3, C-reactive protein/CRP, erythrocyte sedimentation rate/ESR, other laboratory tests), vital signs, physical examination, adverse events, routine safety laboratory tests (hematology, serum chemistry), urinalysis, serum immunoglobulins, peripheral blood B and T cells, epratuzumab serum levels (analyzed by sponsor), and human anti-human (epratuzumab) antibody titers (HAHA; analyzed by sponsor).

Human anti-human (epratuzumab) antibody assay

The sponsor's HAHA test is a competitive ELISA assay, where the capture reagent is epratuzumab and the probe is an anti-epratuzumab-idiotype antibody. The anti-idiotype antibody is an acceptable surrogate for what is reacted against in an immunogenic response by humans against the binding portion of epratuzumab that distinguishes the molecule from other human antibodies (for instance, the framework region that has human amino acid sequences). Test results are derived from

Table 1

Number of patients with B-level disease activity at study entry in each BILAG body system

Body system	Number of patients	Contributing signs/symptoms* (number of patients)
I. General/ constitutional	3	Fatigue/malaise/lethargy (3)
		Anorexia/nausea/vomiting (2)
		Unintentional weight loss > 5% (1)
II. Mucocutaneous	13	Malar erythema (11)
		Active localized discoid lesions (2)
		Mild maculopapular eruption (1)
III. Neurological	0	
IV. Musculoskeletal	2	Arthritis (2)
V. CV/Respiratory	2	Dyspnea (2)
		Pleuropericardial pain (2)
VI. Vasculitis	5	Minor cutaneous vasculitis (nailfold/digital vasculitis, purpura, urticaria) (5)
VII. Renal	0	
VIII. Hematology	1	Anemia (hemoglobin < 11 g/dL) (1)

*Signs and symptoms that contributed to the B-level disease activity according to BILAG rules.

an eight-point standard curve with varying dilutions of anti-idiotype antibody in bovine serum albumin. Patient serum samples are diluted 1:2 with bovine serum albumin and assayed in triplicate. The anti-idiotype standard curve is used to determine the presence of HAHA in unknown samples. An acceptable assay is based on linear regression parameters that must be met to define a valid assay.

Statistical analyses

The primary assessment of disease activity compared post-treatment BILAG results with those at study entry, using total BILAG scores for overall assessment and letter grade categories to assess the level of disease activity within each body system. Adverse events and safety laboratory tests were graded according to NCI CTC version 3.0 criteria on a 1 to 4 scale for toxicity (1, mild; 2, moderate; 3, severe; 4, life threatening). All analyses of efficacy, safety, tolerance, immunogenicity, pharmacokinetics, and pharmacodynamics used descriptive statistics. Wilcoxon signed rank test was used to assess the statistical significance of changes in total BILAG scores compared to their baseline values. All statistical tests used a significance level of 0.05.

Table 2

Number of patients with C-level disease activity at study entry in each BILAG body system

Body system	Number of patients	Contributing signs/symptoms* (number of patients)
I. General/ Constitutional	11	Fatigue/malaise/lethargy (10)
		Anorexia/nausea/vomiting (1)
		Lymphadenopathy/splenomegaly (1)
		Pyrexia (documented) (1)
II. Mucocutaneous	1	Mild alopecia (1)
III. Neurological	10	Episodic migrainous headaches (8)
		Severe, unremitting headache (2)
IV Musculoskeletal	11	Arthralgia (10)
		Myalgia (9)
		Improving arthritis (1)
V. CV/ Respiratory	2	Dyspnea (1)
		Pleuropericardial pain (1)
VI. Vasculitis	4	Raynaud's (3)
		Livido reticularis (1)
VII. Renal	4	Mild/stable proteinuria (4)
VIII. Hematology	11	Lymphocytopenia (< 1500 cells/μl) (10)
		Evidence of circulating anticoagulant (1)
		Decreased platelets (< 150,000/μl) (1)

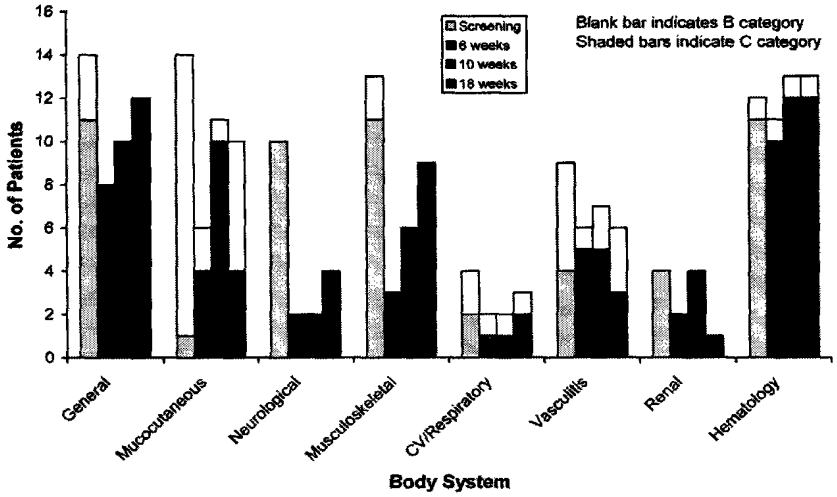
*Signs and symptoms that contributed to the C-level disease activity according to BILAG rules.

Results

Demographics and patient characteristics at study entry

A total of 14 Caucasian patients (13 females and 1 male; 23 to 53 years old, median age 40 years) were enrolled. At study entry, the patients had been initially diagnosed with SLE 1 to 19 years (median 10 years) earlier and were receiving corticosteroids ($n = 13$, 1 to 12 mg/day prednisolone) plus immunosuppressives ($n = 11$, including 50 to 200 mg/day azathioprine, $n = 9$; 20 mg/day methotrexate, $n = 2$; 2 g/day mycophenalate mofetil, $n = 1$), and antimalarials ($n = 6$, 200 to 600 mg/day hydroxychloroquine). All patients had positive ANA at study entry (titers of 80:1 to 5,120:1), and 5 patients (36%) had positive anti-dsDNA antibody levels (> 10 U/ml). Ten patients (71%) had ESR values that were elevated (> 15 mm/h) and 4 patients (29%) had raised CRP levels (> 0.5 mg/dl), while only 3 patients (21%) had C3 levels that were borderline low or decreased (< 90 mg/dl), and no patient had

Figure 1



Frequency comparison of BILAG B- and C-level activities for each body system at screening, 6, 10 and 18 weeks.

positive direct Coombs' or serum haptoglobulin levels elevated above borderline.

All patients had total BILAG scores of 6 to 12 (median 10) at study entry. No patient had A-level disease activity in any body system, 13 patients had B-level disease activity in at least one body system (2 with three Bs, 9 with 2 Bs, 2 with one B) and one patient had only C-level activities. B-level disease occurred primarily in the mucocutaneous, vasculitis, and general/constitutional body systems, with no B-level disease activity in the neurological or renal systems (Table 1), while C-level disease occurred primarily in the general/constitutional, musculoskeletal, hematological and neurological body systems (Table 2). The actual signs and symptoms at study entry that contributed to the B-level disease activity according to the BILAG rules are also summarized in Table 1, while those contributing to C-level disease activity are summarized in Table 2.

Study drug administration

Twelve of the 14 patients (86%) completed all 4 infusions of 360 mg/m^2 epratuzumab as scheduled, while one patient with sleepiness attributed to premedication IV antihistamines prematurely terminated the first infusion but subsequently completed all 3 remaining infusions without further event, and one patient completed the first two infusions, but discontinued further infusions after development of herpes zoster, which responded to antivirals. The infusions were well tolerated, with a median infusion time of 32 minutes (23 to 86 minutes), and with infusion reactions in 6 patients all limited to occurrences of transient, mild (grade 1 NCI toxicity) adverse events (flu-like symptoms, tracheitis/throat ache, $n = 2$; arthralgia/myalgia, fever, fatigue, nausea, headache, chills, or rash, $n = 1$).

Post-treatment evaluations and follow-up

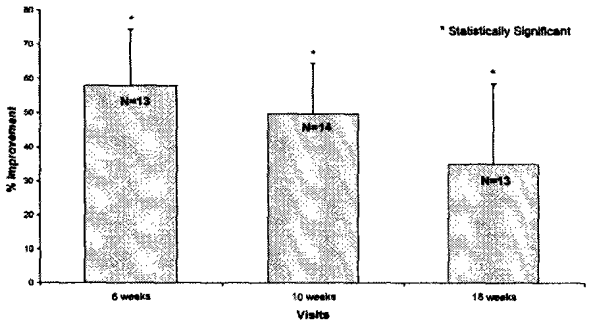
All patients remained in the study through the 18-week post-treatment evaluation period. One patient had a late 18-week visit that fell within the 32-week time frame and the corresponding data were hence re-assigned to the 32-week visit. The single patient who did not complete all 4 infusions continued to receive post-treatment evaluations beginning at the 10-weeks follow-up visit. Except for the aforementioned deviations, all patients received post-treatment evaluations at 6, 10, and 18 weeks. One patient was lost to follow-up after 18 weeks, while 13 patients returned for the final 32-week evaluations (8 patients as scheduled, 5 with a delayed visit between 42 to 82 weeks).

BILAG treatment response

The effect of epratuzumab on clinical manifestations was evaluated at 6, 10, and 18 weeks using numerical total BILAG scores as well as categorical scores. The compositions of B- and C-level activities improved after treatment, primarily in the general, mucocutaneous and musculoskeletal systems (Figure 1). Improvement in C-level activity was also observed in the neurological and renal domains. Improvements in the general, mucocutaneous, neurological and musculoskeletal systems occurred earlier compared to the cardiovascular/respiratory, vasculitic and renal systems (Figure 2). However, the limited number of patients with manifestations in each of these systems precludes a definitive determination of preferential effects. In terms of changes in the total BILAG score, statistically significant improvement was observed at 6, 10, and 18 weeks (Figure 3). Additionally, a substantial proportion of patients showed 50% or more improvement in total BILAG score at weeks 6, 10, and 18 (77%, 71% and 38%, respectively). At the final 32-week evaluation, statistically significant

Figure 2

Level of Improvement	6 weeks	10 weeks	18 weeks
Pts with Decreased Scores	100% (13/13)	100% (14/14)	92% (12/13)
Pts with Decreases ≥ 50%	77% (10/13)	71% (10/14)	38% (5/13)



Overall frequency and mean improvement of total disease activity as measured by the total BILAG score at 6, 10 and 18 weeks.

improvement in total BILAG score continued to be observed, with 15% of the patients achieving 50% or more improvement.

In a separate analysis, the total number of patients who achieved BILAG improvements in the particular domains at 6, 10 and 18 weeks of follow-up are summarized in Table 3. This indicates that the most characteristic BILAG domains, as also seen in Figure 2, were more likely to respond, although the duration of response was very similar throughout the domains. In fact, deterioration in BILAG categorical scores compared to baseline was infrequently seen during the study (Table 4). Only two patients (14%) showed worsening of hematological

Table 3

Number of patients with improvement from baseline BILAG B- and C-level activities

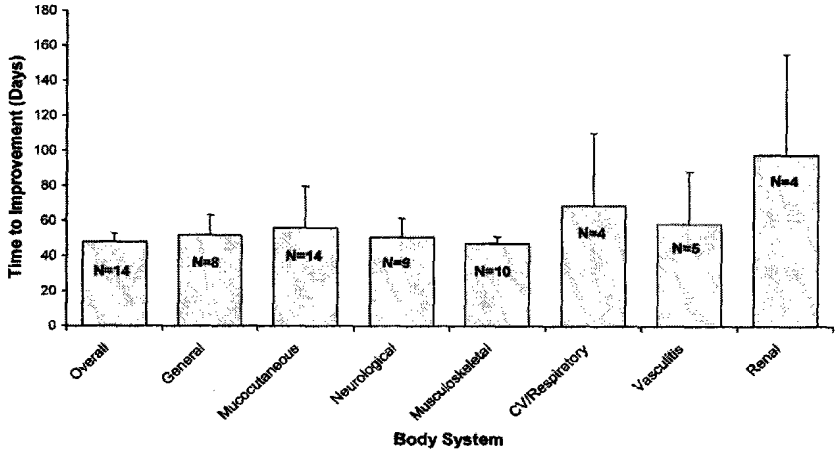
BILAG body system	6 weeks[a]	10 weeks	18 weeks
General ($N = 14$)[b]	6 (43%)	5 (36%)	2 (14%)
Mucocutaneous ($N = 14$)	11 (79%)	8 (57%)	6 (43%)
Neurological ($N = 10$)	7 (70%)	8 (80%)	6 (60%)
Musculoskeletal ($N = 13$)	9 (69%)	7 (54%)	4 (31%)
CV/Respiratory ($N = 4$)	3 (75%)	3 (75%)	3 (75%)
Vasculitis ($N = 9$)	4 (44%)	3 (33%)	3 (33%)
Renal ($N = 4$)	2 (50%)	1 (25%)	3 (75%)
Hematology ($N = 12$)	0 (0 %)	0 (0 %)	0 (0 %)
Overall[c] ($N = 14$)	13 (93%)	14 (100%)	13 (93%)

[a]Twenty-four hours after fourth infusion. [b]N = number of patients with involvement in a particular body system at entry. [c]As applied to any BILAG body system.

parameters (lymphocytopenia), one starting at 6 weeks and the other at 18 weeks. Another patient manifested renal (mild proteinuria) deterioration at 10 weeks. Overall, at week 18, 3 patients (21%) had a deteriorated BILAG assessment in at least one body system compared to baseline.

An additional analysis was performed to determine the durability of resolution of certain B- and C-level activities (Table 5). Although in a number of patients, B- and C-level activities resolved persistently, the heterogeneity of patients' manifesta-

Figure 3



Mean time to improvement of each BILAG body stystem. Mean time to improvement (in days) of each BILAG body system during the follow-up of the study (N denotes the number of patients available for analysis for each body system). Since the first evaluation was scheduled for 6 weeks, the earliest time to improvement is at least 42 days.

tions again precluded the identification of a preferential response profile to the drug.

Safety

During or following treatment, a total of ten patients reported adverse events. As reported above, six had mild, transient, infusional reactions and one patient experienced somnolence following antihistamine medication. Subsequently, five patients had infections (including herpes zoster, otitis media, *Helicobacter pylori*-associated gastritis, vaginitis/vaginal candidiasis, cystitis, and tonsillitis) that resolved with appropriate treatment, and one patient had spinal contusion from a traffic accident. Standard safety laboratory tests showed no consistent pattern of change from baseline, and infrequent post-treatment increases in NCI CTC v3.0 toxicity grades for these laboratory tests were all limited to changes of one grade level except for one patient with an increase in lymphocytes from grade 1 to grade 3, and another from grade 0 to grade 3 (Table 6).

Pharmacokinetics and immunogenicity

Of the 14 patients, serum samples for analysis of pharmacokinetics and immunogenicity (HAHA) by ELISA assay were collected in a limited number of patients post-treatment at 6 weeks (n = 12), 10 weeks (n = 7) and 18 weeks (n = 7). Epratuzumab serum levels were measurable in all available samples through at least 10 weeks post-treatment and were still detectable above the 0.5 μg/ml assay limit in 5/7 samples evaluated at 18 weeks, with median values of 120 μg/ml (range 49 to 350) at 6 weeks, 48 μg/ml (range 31 to 138) at 10 weeks, and 8.3 μg/ml (range 1.82 to 25) at 18 weeks. Figure 4 shows the individual measurements over time. There was a single sample showing 1.42 μg/ml at 32 weeks. HAHA analysis gave no evidence of immunogenicity, with all post-treatment values either remaining below the 50 ng/ml sensitivity of the assay or not increased from baseline values prior to treatment.

Table 4

Number of patients with deteriorating BILAG activities from baseline

BILAG body system (N = 14)[a]	6 weeks[b]	10 weeks	18 weeks
General	0 (0 %)	0 (0 %)	0 (0 %)
Mucocutaneous	0 (0 %)	0 (0 %)	0 (0 %)
Neurological	0 (0 %)	0 (0 %)	0 (0 %)
Musculoskeletal	0 (0 %)	0 (0 %)	0 (0 %)
CV/Respiratory	0 (0 %)	0 (0 %)	1 (7 %)
Vasculitis	0 (0 %)	0 (0 %)	0 (0 %)
Renal	0 (0 %)	1 (7 %)	0 (0 %)
Hematology	1 (7 %)	1 (7 %)	2 (14%)
Overall[c]	1 (7 %)	2 (14%)	3 (21%)

[a]N = total number of patients. [b]Twenty-four hours after fourth infusion. [c]As applied to any BILAG body system.

Immunology laboratory tests

Table 7 shows that at the first evaluation after treatment, mean B cell levels decreased by 35% and persisted at these levels on subsequent evaluations (Figure 5), with no evidence of onset of recovery by the final study evaluation at 32 weeks (6 months post-treatment). In contrast, there does not appear to be any consistent pattern of decreases/increases in T cell levels or serum levels of IgG, IgA, or IgM following treatment (Table 7).

Although all 14 patients had measurable ANA titers (1:80 to 1:5,120) at study entry, no patient had consistent post-treatment decreases, including evaluations at 32 weeks (6 months post-treatment) follow-up (8 patients had no changes at any evaluation, 5 doubled their baseline titers at one or more evaluations, and one patient had an isolated decrease at one evaluation). Five patients had elevated anti-dsDNA antibodies (10 to 123 U/ml) at study entry, but none had any decreased post-treatment values (2 patients had no significant changes, and 3 had increases at one or more evaluations). C3 levels that were decreased or borderline for 3 patients at study entry remained virtually unchanged post-treatment, as did mean C3 values for all patients.

Table 5

Number of patients in each BILAG body system with resolution of baseline B- and C-level disease activities

Body system	B level	C level
General	3/3 (100%)	0/11 (0%)
Mucocutaneous	4/13 (31%)	0/1 (0%)
Neurological	0/0	2/10 (20%)
Musculoskeletal	1/2 (50%)	1/11 (9%)
CV/Respiratory	0/2 (0%)	2/2 (100%)
Vasculitis	2/5 (40%)	0/4 (0%)
Renal	0/0	2/4 (50%)
Hematology	0/1 (0%)	0/11 (0%)

Resolution is defined as post-treatment improvement of baseline disease activity level by at least one category level (B to C, D, or E; C to D or E) at one or more evaluations up to 18 weeks, with no categorical deterioration from the baseline activity level prior to improvement, and no reversion to the baseline activity level once any improvement has occurred. Additionally note that 3 patients with multiple BILAG B involvement at baseline had completely resolved all B-level disease activities by 18 weeks.

Table 6

Post-treatment increases in NCI CTC v3.0 toxicity grades from baseline values

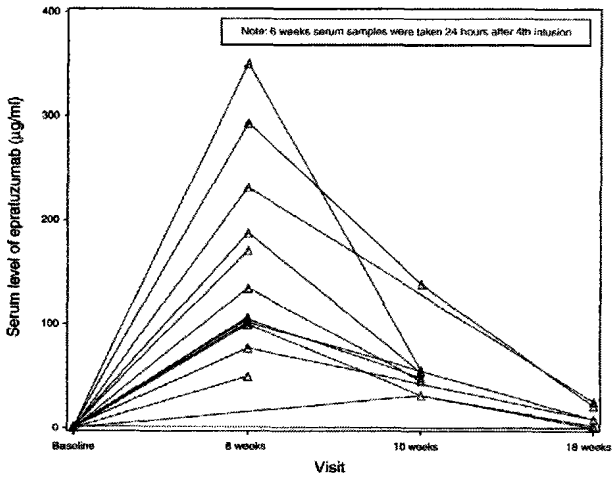
Labparameter	No increase	Toxicity increase	
		1 grade	2–3 grades
Hematology			
Hemoglobin	10	4	0
Platelets	12	2	0
WBC	11	3	0
ALC	6	6	2
ANC	13	1	0
Chemistry			
Creatinine	10	4	0
Total Bilirubin	14	0	0
Alkaline phosphatase	12	2	0
ALT (SGPT)	9	5	0
AST (SGOT)	10	4	0
GGT	12	2	0

ALC, absolute lymphocyte count, ANC, absolute neutrophil count, ALT, alanine aminotransferase, AST, aspartate aminotransferase, GGT, gamma glutamyl transferase, WBC, white blood cell

Discussion

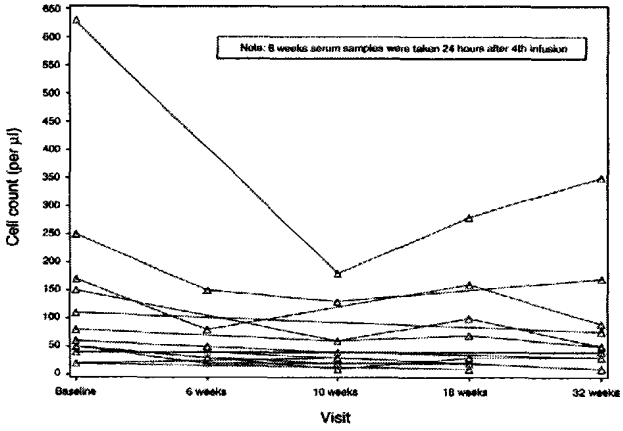
The pathogenesis of SLE remains enigmatic, but a central feature of this disease is the loss of immune tolerance and enhanced B cell activity. Although the number of B cells in the peripheral blood is often decreased, those that are present show characteristic alterations and have abnormal phenotypes indicative of activation [5,47]. Therefore, B cell depletion is an attractive therapeutic strategy for patients with SLE. The availability of the chimeric anti-CD20 antibody rituximab (Rituxan® Genentech, South San Francisco, CA, USA; Biogen Idec, Boston, MA, USA) made it possible to test this hypothesis.

Figure 4



Serum levels of epratuzumab as detected by ELISA in the patients during the study.

Figure 5



Follow-up of peripheral B cell levels during the study among individual study patients.

Initially, Isenberg and coworkers [19] treated 6 patients with active and otherwise refractory SLE (median BILAG score 14, range 9 to 27) with rituximab given in 500 mg doses 2 weeks apart with 2 doses of 750 mg iv cyclophosphamide and oral prednisolone cover (30 or 60 mg for 5 days). The treatment was safe and well tolerated, B cell depletion occurred, and BILAG total scores improved at 6 months (median 6, range 3 to 8). Looney and colleagues [6] initiated an open-label rituximab study of 17 patients with SLE (≥ 6 systemic lupus activity measurement, SLAM score) who were treated with either one 100 mg/m^2 dose, one 375 mg/m^2 dose, or four 375 mg/m^2 doses. Oral prednisone (40 mg for two doses) also was administered. B cell decreases were variable, with a 35% mean decrease persisting over the 6-month observation period, and clinical efficacy was demonstrated in patients with B cell depletion. Less than 6/17 of their patients developed human anti-chimeric antibody (HACA) at a level higher than or equal to 100 ng/ml when treated with this protocol.

All of these studies and case reports have so far been of short duration [7,48]. Usually, the B cell depletion in SLE is profound, as in patients with NHL, but shorter lasting. Therefore, it is very likely that cyclical therapy will be needed to provide long-term benefit for patients with SLE. While the immunogenicity of rituximab has not been clinically important (HACA < 1%) for the management of patients with NHL, approximately 4% of patients with rheumatoid arthritis developed HACA and 8% to 10% with SLE did so also, in spite of being

Table 7

Post-treatment changes of lymphocytes and immunoglobulins

	Baseline values and post-treatment percent change from baseline (mean ± SD)				
	Baseline	6 weeks	10 weeks	18 weeks	32 weeks
Lymphocytes	$N = 14$	$N = 6$	$N = 8$	$N = 9$	$N = 11$
B cells	123 ± 160 cells/μl	-35% ± 23%	-41% ± 41%	-34% ± 23%	-44% ± 21%
T cells	744 ± 554 cells/μl	+16% ± 80%	+28% ± 78%	+47% ± 109%	+17% ± 69%
Immunoglobulins		$N = 12$	$N = 14$	$N = 10$	$N = 11$
IgG	1,252 ± 355 mg/dl	+3% ± 8%	+5% ± 13%	+5% ± 9%	1% ± 13%
IgA	226 ± 94 mg/dl	+3% ± 11%	+8 ± 13%	+5% ± 12%	+10% ± 20%
IgM	117 ± 73 mg/dl	-12% ± 18%	-1% ± 23%	-6% ± 19%	-9% ± 9%

SD, standard deviation.

treated with various doses of steroids and/or cytotoxic agents in combination with rituximab. Thus, a less immunogenic antibody (for example, a human or humanized form) is likely needed in the management of patients with autoimmune diseases, since it is expected that repeated dosing will be required in patients with such chronic diseases.

This initial study demonstrated that 360 mg/m^2 epratuzumab, a humanized CD22-specific monoclonal antibody, administered every other week for a total of 4 doses was safe and well-tolerated in SLE patients, with few significant adverse events, alterations of standard safety laboratory tests, and no evidence of immunogenicity. In addition to the minimal infusion reactions, the ability to complete an infusion within approximately 0.5 to 1 hour and the lack of immunogenicity are also likely to be more important treatment considerations in autoimmune diseases, as mentioned previously.

With this dosing schedule, virtually every patient with moderate disease activity (total BILAG score of 6 to 12) demonstrated symptomatic improvement using BILAG total scores. The BILAG total score results indicate that 77% of the patients achieved a ≥ 50% decrease in their overall disease activity at 6 weeks follow up. Furthermore, most patients (92%) continued to show reduced disease activity for at least 18 weeks, and even 38% showed a sustained response with BILAG reductions of 50% or more compared to study entry. Since this first study considered moderately active lupus patients with BILAG total scores of 6 to 12, the resulting heterogeneity precludes the identification of any preferential effect on one or the other BILAG domains as shown from different perspectives of efficacy analysis.

In addition to treating mild BILAG C-level symptoms, epratuzumab immunotherapy reduced all BILAG B-level activity in the majority of patients presenting with more serious disease, including patients with B-level activity in several body systems. The current data limit the conclusions that can be drawn regarding therapeutic effects for some systems, such as B-level disease in the neurological and renal systems, and only one case of lymphopenia in the hematological system showed improvement. In spite of small numbers, CD22-immunotherapy with epratuzumab appeared to be effective for treating disease in many of the other body/organ systems.

Although the biweekly dosing schedule used in this study demonstrated apparent activity, the serum levels of antibody measured here appear to be less than those in studies of NHL, where a weekly schedule of dose administrations has shown antitumor activity [42-44]. Hence, other dosing schedules in future clinical trials are warranted to assess the effects of increasing the serum levels of epratuzumab.

Compared to the complete depletion of B cells observed with rituximab, a long-lasting (at least 6 months, the last observation time) decrease of about 35% to 40% occurred with epratuzumab, with no apparent changes in T cells or immunoglobulin levels. As discussed earlier, the attractiveness of CD22 as a molecular target for therapy in SLE extends beyond the capability of epratuzumab to modestly decrease peripheral blood levels of B cells. CD22 is a cell surface receptor that is a member of the sialioadhesion family and an inhibitory co-receptor of BCR [34]. *In vitro* studies demonstrated that epratuzumab binding can induce CD22 phosphorylation [49] , and the current data from this study suggest that epratuzumab could potentially mediate direct pharmacological effects by negatively regulating certain hyperactive B cells. This hypothesis now needs to be tested. Interestingly, over the period of this study, patients clinically improved without clear evidence of reduction in ANA or anti-dsDNA titers. Similar observations have been reported with rituximab [19] , further supporting the hypothesis that targeted therapy impacting the hyperactive B cell compartment may be successful without needing to completely deplete the broader B cell population.

Conclusion

This initial experience in lupus patients with mild to moderate symptoms demonstrated that 4 doses of 360 mg/m^2 epratuzumab immunotherapy are safe and well tolerated when infused within one hour, with consistent improvement observed in all patients for at least 12 weeks in the presence of modestly decreased (about 35%) peripheral B cell levels, and with no evidence of HAHA. Although this was an open-label study, consistent improvement was observed in all patients for at least 12 weeks, and there was reduction or elimination of disease activity across most body systems, regardless of the extent or the severity of the presenting disease activity. The duration of response was very heterogeneous for different BILAG domains, precluding firm conclusions at this time. As such, these results support conducting longer-term multicenter randomized controlled studies, which are now underway to examine the effects of epratuzumab in broader patient populations with autoimmune disease.

Competing interests

TD, JK, and GRB declare research funding for this study provided by Immunomedics, Inc. WAW, NT, and DMG have employment and financial interests (stock) in Immunomedics, Inc., whichowns the antibody tested in this paper.

Authors' contributions

All authors contributed to data interpretation and the final manuscript. TD and GRB were the principal investigators and were responsible for coordinating the study, while JK participated in patient selection and directed all patient related study procedures. DMG, TD and WAW designed the clinical trial protocol, and NT was responsible for data management and statistical analysis. TD and JK contributed equally to this work.

Acknowledgements

The authors acknowledge the patients who agreed to participate in this study. This study was supported in part by the Sonderforschungsbereich 650 (TD, GRB), and by Immunomedics, Inc. We thank Vibeke Strand, MD, for her helpful comments for improving the manuscript.

References

1. Snaith ML, Isenberg DA: **Systemic lupus erythematosus and related disorders.** In *Oxford Textbook of Medicine* 3rd edition. Edited by: Weatherall DJ, Ledingham JGG, Warrell DA. Oxford: Oxford University Press; 1996:3017-3027.
2. Jacobson DL, Gange SJ, Rose NR, Graham NM: **Epidemiology and estimated population burden of selected autoimmune diseases in the United States.** *Clin Immunol Immunopathol* 1997, **84**:223-243.
3. Datta SK: **Production of pathogenic antibodies: cognate interactions between autoimmune T and B cells.** *Lupus* 1998, **7**:591-596.
4. Llorente L, Richaud-Patin Y, Wijdenes J, Alcocer-Varela J, Maillot MC, Durand-Gasselin I, Fourrier BM, Galanaud P, Emilie D: **Spontaneous production of interleukin-10 by B lymphocytes and monocytes in systemic lupus erythematosus.** *Eur Cytokine Netw* 1993, **4**:421-427.
5. Jacobi AM, Odendahl M, Reiter K, Bruns A, Burmester GR, Radbruch A, Valet G, Lipsky PE, Dörner T: **Correlation between circulating CD27high plasma cells and disease activity in patients with systemic lupus erythematosus.** *Arthritis Rheum* 2003, **48**:1332-1342.
6. Looney RJ, Anolik JH, Campbell D, Felgar RE, Young F, Arend LJ, Sloand JA, Rosenblatt J, Sanz I: **B cell depletion as a novel treatment for systemic lupus erythematosus: a phase I/II dose-escalation trial of rituximab.** *Arthritis Rheum* 2004, **50**:2580-2589.
7. Sfikakis PP, Boletis JN, Tsokos GC: **Rituximab anti-B cell therapy in systemic lupus erythematosus: pointing to the future.** *Curr Opin Rheumatol* 2005, **17**:550-557.
8. Rajagopalan S, Zordan T, Tsokos GC, Datta SK: **Pathogenic anti-DNA autoantibody-inducing T helper cell lines from patients with active lupus nephritis: isolation of CD4-8- T helper cell lines that express the gamma delta T-cell antigen receptor.** *Proc Natl Acad Sci USA* 1990, **87**:7020-7024.9.
9. de Vos AF, Fukushima A, Lobanoff MC, Vistica BP, Lai JC, Grivel JC, Wawrousek EF, Whitcup SM, Gery I: **Breakdown of tolerance to a neo-self antigen in double transgenic mice in which B cells present the antigen.** *J Immunol* 2000, **164**:4594-4600.
10. Roth R, Gee RJ, Mamula MJ: **B lymphocytes as autoantigen-presenting cells in the amplification of autoimmunity.** *Ann NY Acad Sci* 1997, **815**:88-104.
11. Demaison C, Chastagner P, Theze J, Zouali M: **Somatic diversification in the heavy chain variable region genes expressed by human autoantibodies bearing a lupus-associated nephritogenic anti-DNA idiotype.** *Proc Natl Acad Sci USA* 1994, **91**:514-518.
12. Manheimer-Lory AJ, Zandman-Goddard G, Davidson A, Aranow C, Diamond B: **Lupus-specific antibodies reveal an altered pattern of somatic mutation.** *J Clin Invest* 1997, **100**:2538-2546.
13. Grammer AC, Slota R, Fischer R, Gur H, Girschick H, Yarboro C, Illei GG, Lipsky PE: **Abnormal germinal center reactions in systemic lupus erythematosus demonstrated by blockade of CD154-CD40 interactions.** *J Clin Invest* 2003, **112**:1506-1520.
14. Andrews BS, Eisenberg RA, Theofilopoulos AN, Izui S, Wilson CB, McConahey PJ, Murphy ED, Roths JB, Dixon FJ: **Spontaneous murine lupus-like syndromes. Clinical and immunopathological manifestations in several strains.** *J Exp Med* 1978, **148**:1198-1215.
15. Shlomchik MJ, Madaio MP, Ni D, Trounstein M, Huszar D: **The role of B cells in lpr/lpr-induced autoimmunity.** *J Exp Med* 1994, **180**:1295-1306.
16. Chan O, Shlomchik MJ: **A new role for B cells in systemic autoimmunity: B cells promote spontaneous T cell activation in MRL-lpr/lpr mice.** *J Immunol* 1998, **160**:51-59.
17. Chan OT, Hannum LG, Haberman AM, Madaio MP, Shlomchik MJ: **A novel mouse with B cells but lacking serum antibody reveals an antibody-independent role for B cells in murine lupus.** *J Exp Med* 1999, **189**:1639-1648.
18. Dörner T, Radbruch A: **Selecting B cells and plasma cells to memory.** *J Exp Med* 2005, **201**:497-499.
19. Leandro MJ, Edwards JC, Cambridge G, Ehrenstein MR, Isenberg DA: **An open study of B lymphocyte depletion in systemic lupus erythematosus.** *Arthritis Rheum* 2002, **46**:2673-2677.
20. Eisenberg R, Albert D, Stansberry J, Tsai D, Kolasinski S, Khan S: **A phase I trial of B cell depletion with anti-CD20 monoclonal antibody (rituximab) in the treatment of systemic lupus erythematosus [abstract].** *Arthritis Res Ther* 2003, **5(Suppl 3)**:S9-10.
21. Edwards JC, Szczepanski L, Szechinski J, Filipowicz-Sosnowka A, Emery P, Close DR, Stevens RM, Shaw T: **Efficacy of B cell-targeted therapy with rituximab in patients with rheumatoid arthritis.** *N Engl J Med* 2004, **350**:2572-2581.
22. Van Vollenhoven RF, Gunnarsson I, Welin-Henriksson E, Sundelin B, Jacobson SH, Kareskog L: **A 4-week course of rituximab plus cyclophosphamide in severe SLE: promising results in 9 patients who failed conventional immunosuppressive therapy. EULAR 2004 [abstract].** *Ann Rheum Dis* **63(Suppl 1)**:863HH.
23. Tedder TF, Tuscano J, Sato S, Kehrl JH: **CD22, a B lymphocyte-specific adhesion molecule that regulates antigen receptor signaling.** *Annu Rev Immunol* 1997, **15**:481-504.
24. Peaker CJ, Neuberger MS: **Association of CD22 with the B cell antigen receptor.** *Eur J Immunol* 1993, **23**:1358-1363.
25. Nath D, van der Merwe PA, Kelm S, Bradfield P, Crocker PR: **The amino-terminal immunoglobulin-like domain of sialoadhesin contains the sialic acid binding site. Comparison with CD22.** *J Biol Chem* 1995, **270**:26184-26191.

26. Sgroi D, Koretzky GA, Stamenkovic I: **Regulation of CD45 engagement by the B cell receptor CD22.** *Proc Natl Acad Sci USA* 1995, **92**:4026-4030.
27. Kelm S, Pelz A, Schauer R, Filbin MT, Tang S, de Bellard ME, Schnaar RL, Mahoney JA, Hartnell A, Bradfield P, *et al.*: **Sialoadhesin, myelin-associated glycoprotein and CD22 define a new family of sialic acid-dependent adhesion molecules of the immunoglobulin superfamily.** *Curr Biol* 1994, **4**:965-972.
28. Clark EA: **CD22, a B cell-specific receptor, mediates adhesion and signal transduction.** *J Immunol* 1993, **150**:4715-4718.
29. Engel P, Nojima Y, Rothstein D, Zhou LJ, Wilson GL, Kehrl JH, Tedder TF: **The same epitope on CD22 of B lymphocytes mediates the adhesion of erythrocytes, T and B lymphocytes, neutrophils, and monocytes.** *J Immunol* 1993, **150**:4719-4732.
30. Powell LD, Varki A: **I-type lectins.** *J Biol Chem* 1995, **270**:14243-14246.
31. Otipoby KL, Andersson KB, Draves KE, Klaus SJ, Farr AG, Kerner JD, Perlmutter RM, Law CL, Clark EA: **CD22 regulates thymus-independent responses and the lifespan of B cells.** *Nature* 1996, **384**:634-637.
32. Poe JC, Fujimoto M, Jansen PJ, Miller AS, Tedder TF: **CD22 forms a quaternary complex with SHIP, Grb2, and Shc. A pathway for regulation of B lymphocyte antigen receptor-induced calcium flux.** *J Biol Chem* 2000, **275**:17420-17427.
33. O'Keefe TL, Williams GT, Batista FD, Neuberger MS: **Deficiency in CD22, a B cell-specific inhibitory receptor, is sufficient to predispose to development of high affinity autoantibodies.** *J Exp Med* 1999, **189**:1307-1313.
34. Sato S, Tuscano JM, Inaoki M, Tedder TF: **CD22 negatively and positively regulates signal transduction through the B lymphocyte antigen receptor.** *Semin Immunol* 1998, **10**:287-297.
35. Nadler MJ, McLean PA, Neel BG, Wortis HH: **B cell antigen receptor-evoked calcium influx is enhanced in CD22-deficient B cell lines.** *J Immunol* 1997, **159**:4233-4243.
36. Nitschke L, Carsetti R, Ocker B, Kohler G, Lamers MC: **CD22 is a negative regulator of B cell receptor signaling.** *Curr Biol* 1997, **7**:133-143.
37. Chen J, McLean PA, Neel BG, Okunade G, Shull GE, Wortis HH: **CD22 attenuates calcium signaling by potentiating plasma membrane calcium-ATPase activity.** *Nat Immuno* 2004, **5**:651-657.
38. Pawlak-Byczkowska EJ, Hansen HJ, Dion AS, Goldenberg DM: **Two new monoclonal antibodies, EPB-1 and EPB-2, reactive with human lymphoma.** *Cancer Res* 1989, **49**:4568-4577.
39. Stein R, Belisle E, Hansen HJ, Goldenberg DM: **Epitope specificity of the anti-(B cell lymphoma) monoclonal antibody, LL2.** *Cancer Immunol Immunother* 1993, **37**:293-298.
40. Leung SO, Goldenberg DM, Dion AS, Pellegrini MC, Shevitz J, Shih LB, Hansen HJ: **Construction and characterization of a humanized, internalizing, B cell (CD22)-specific, leukemia/lymphoma antibody, LL2.** *Mol Immunol* 1995, **32**:1413-1427.
41. Gada P, Hernandez-Ilizaliturri F, Repasky EA, Czuczman MS: **Epratuzumab's predominant antitumor activity *in vitro/in vivo* against non-Hodgkin's lymphoma (NHL) is via antibody-dependent cellular cytotoxicity (ADCC) [abstract].** *Blood* 2002, **100/11**:353a.
42. Leonard JP, Coleman M, Ketas JC, Chadburn A, Ely S, Furman RR, Wegener WA, Hansen HJ, Ziccardi H, Eschenberg M, *et al.*: **Phase I/II trial of epratuzumab (humanized anti-CD22 antibody) in indolent non-Hodgkin's lymphoma.** *J Clin Oncol* 2003, **21**:3051-3059.
43. Leonard JP, Coleman M, Ketas JC, Chadburn A, Furman R, Schuster MW, Feldman EJ, Ashe M, Schuster SJ, Wegener WA, *et al.*: **Epratuzumab, a humanized anti-CD22 antibody, in aggressive non-Hodgkin's lymphoma: phase I/II clinical trial results.** *Clin Cancer Res* 2004, **10**:5327-5334.
44. Perotti B, Doshi S, Chen D, Gayko U, Leonard JP, Wegener WA, Goldenberg DM, Cesano A: **Pharmacokinetics of epratuzumab administered as a single agent or in combination with rituximab in patients with B cell NHL [abstract].** *Proc Am Soc Clin Oncol* 2003, **22**:.
45. Hay EM, Bacon PA, Gordon C, Isenberg DA, Maddison P, Snaith ML, Symmons DPM, Viner N, Zoma A: **The BILAG index: a reliable and valid instrument for measuring clinical disease activity in systemic lupus erythematosus.** *Quart J Med* 1993, **86**:447-458.
46. Isenberg DA, Gordon C: **From BILAG to BLIPS. Disease activity assessment in lupus: past, present and future.** *Lupus* 2000, **9**:651-654.
47. Odendahl M, Jacobi A, Hansen A, Feist E, Hiepe F, Burmester GR, Lipsky PE, Radbruch A, Dörner T: **Disturbed peripheral B lymphocyte homeostasis in systemic lupus erythematosus.** *J Immunol* 2000, **165**:5970-5979.
48. Silverman GJ: **Anti-CD20 therapy in systemic lupus erythematosus: a step closer to the clinic.** *Arthritis Rheum* 2005, **52**:371-377.
49. Carnahan J, Wang P, Kendall R, Chen C, Hu S, Boone T, Juan T, Talvenheimo J, Montestruque S, Sun J, *et al.*: **Epratuzumab, a humanized monoclonal antibody targeting CD22: characterization of *in vitro* properties.** *Clin Cancer Res* 2003, **9**:3982s-3990s.